Remarks

I. Status of the Claims

Upon entry of the foregoing amendment, claims 86-123, 125-149, and 151-165 are pending in the application, with claims 86 and 87 being the independent claims. Claims 133-157 are withdrawn from consideration as being directed to a non-elected invention. Claims 86, 87, and 106 are amended. New claims 163-165 are sought to be added. Claims 124 and 150 are canceled. Claims 1-85 were previously canceled. These changes are believed to introduce no new matter, and their entry is respectfully requested.

II. The Amendments

The pending claims are directed to methods for producing a recombinant antibody having increased Fc mediated cellular cytotoxicity (*e.g.*, claim 86) or, alternatively, increased Fc receptor binding affinity (*e.g.*, claim 87).

Support for the amendments and new claims can be found *inter alia* in the disclosure as follows:

CLAIM	SUPPORT
86, 87	See, for example, page 7, line 33 through page 8, line 1; page 17, lines 29-33; page 15, lines 5-8; page 21, line 15 through page 22, line 22; and Examples 3 and 4.
106	See, for example, page 7, line 33 through page 8, line 1; and page 17, lines 29-33.
163	See, for example, page 15, lines 5-8; page 21, line 15 through page 22, line 22; and Examples 3 and 4.
164, 165	See, for example, page 7, lines 14-16; and page 21, lines 15-25.

Accordingly, no new matter is believed to have been added by the amendments, and their entry is respectfully requested.

III. Brief Description of the Invention

The present invention is directed to a method for producing a recombinant antibody having increased Fc mediated cellular cytotoxicity (*e.g.*, claim 86) or, alternatively, increased Fc receptor binding affinity (*e.g.*, claim 87), comprising providing a *mammalian host cell* that expresses a recombinant antibody comprising an *IgG Fc* region containing N-linked oligosaccharides; glycoengineering the host cell so that it has an *altered level of activity of at least one glycoprotein-modifying glycosyltransferase*; culturing the glycoengineered host cell under conditions which permit the production of the recombinant antibody; and isolating the recombinant antibody, wherein the recombinant antibody has increased Fc-mediated cellular cytotoxicity or, alternatively, increased Fc receptor binding affinity compared to the corresponding antibody produced by the same host cell that has not been glycoengineered.

This invention is the result of Applicants' discovery that the oligosaccharides that occur in the Fc region of antibodies, such as IgG, can be engineered, by a variety of methods, to produce non-naturally occurring *variant* oligosaccharide conformations that have been found to dramatically increase the antibody effector function, such as antibody-dependent cellular cytotoxicity (ADCC), as well as the antibody's affinity for Fc receptors.

Typically, there is heterogeneous processing of the core oligosaccharide structures attached at a particular glycosylation site, so that even monoclonal antibodies exist as multiple glycoforms. By engineering the host cells that produce the antibodies to

favor production of an antibody having a variant oligosaccharide conformation in the Fc region, *i.e.*, having a significant increase in the proportion of nonfucosylated oligosaccharide structures, Applicants are able to generate variant glycoforms of the antibody having oligosaccharide conformations that are *not capable of being produced by the host cell absent the glycoengineering* and which exhibit dramatically increased ADCC and Fc receptor binding compared to the corresponding nonglycoengineered antibody.

IV. The Restriction Requirement

Applicants' election with traverse of Group I (Claims 86-132 and 158-160) has been acknowledged and the requirement has been made final. Office Action at page 2.

V. Priority

The Office has alleged that claims 90, 94, 96, 97, 101, 106-108, 114, 127, and 128 are granted priority only as of the filing date of the instant application (August 5, 2003) because the Office contends that they do not have support in the parent applications. Office Action at page 2. Applicants respectfully traverse. It is Applicants' understanding that the alleged lack of support refers to the Office's arguments with respect to the written description and enablement requirements under 35 U.S.C. § 112, first paragraph. Hence, Applicants direct the Office to the detailed discussion herein below showing that the claimed invention is supported by sufficient written description and an enabling disclosure. As such, Applicants respectfully submit that all of the claims of the present application are supported in the parent applications (60/082,581 and 09/294,584) and are therefore entitled to claim the benefit of priority of these applications.

The Office has acknowledged that claims 91-93, 95, 98-100, 102-105, 109-113, 115-126, and 129-162 are entitled to priority of the filing date of the 06/082,581 application (April 20, 1998). *Id.*

VI. The Rejections

A. Rejection Of Claims 86-88, 90-91, 95-98, 100, 102-103, 105-132, and 158-160 Under 35 U.S.C. § 112, First Paragraph--Enablement

At page 3 of the Office Action, the Office has rejected claims 86-88, 90-91, 95-98, 100, 102-103, 105-132, and 158-160 under 35 U.S.C. § 112, first paragraph as allegedly lacking enablement. Applicants respectfully traverse this rejection.

The test for enablement is whether one of ordinary skill in the art, given the disclosure at the time of filing, could make and use the claimed invention without undue experimentation. *See In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). Several factors must be considered when determining whether experimentation is "undue," including predictability in the art, state of the art, presence or absence of working examples, amount of guidance presented, nature of the invention, breadth of the claims, and level of skill in the art. *Id.* "'The key word is "undue," not "experimentation."'" *Id.* at 737 (quoting *In re Angstadt*, 537 F.2d at 504). "The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation." MPEP § 2164.01 (Rev. 3, Aug. 2005) at 2100-193 (citations omitted).

While the Office addressed several of the *Wands* factors in the Office Action, the focus of the rejection appears to be based on the alleged unpredictability in the art and an alleged lack of guidance in the specification. As discussed below, the art is sufficiently predictable to enable the claimed invention and, given the knowledge in the art and the

guidance provided by Applicants' disclosure, one of ordinary skill in the art could practice the full scope of the claimed invention without undue experimentation.

1. The Art is Sufficiently Predictable to Enable the Claimed Invention

The Office states that "[t]he art here involves glycoengineering recombinant antibodies so as to increase Fc-mediated cellular cytotoxicity (ADCC)," and asserts that the art in this field is unpredictable. Office Action at page 4. The Office appears to be basing the assertion of unpredictability in the art on two main grounds: 1) a presumption that ADCC activity must always correlate directly with the levels of bisected hybrid oligosaccharides for there to be predictability with respect to the effects of expression of other glycoprotein-modifying glycosyltransferases on ADCC; and 2) alleged unpredictability associated with the choice of host cell and the differences in glycosylation machinery among cell types. *Id.* at pages 4-6. Applicants respectfully disagree with these assertions for the following reasons.

a) The Effect of Bisecting GlcNAc on Fc-mediated Cytotoxicity is not Controversial

In asserting unpredictability of the art, the Office has presumed that an increase in ADCC must be "due to an increase in the proportion of antibodies having bisected, hybrid glycans as a result of expression of the GnTIII gene." Office Action at page 4. The Office concludes that "a higher ratio of bisected, hybrid to bisected, complex glycans does not always correlate with increased ADCC and may even lead to decreased ADCC," and that "the effects of expression of other glycoprotein-modifying glycosyltransferases ... on increasing ADCC activity is unknown." *Id.* at page 5. The Office cites Shinkawa *et al.*, *J. Biol. Chem.* 278: 3466-73 (2003), for the proposition that "the significance of bisecting GlcNAc in enhancing ADCC is controversial," because

Shinkawa *et al.* "indicates that the absence of fucose in oligosaccharides on IgG1 molecules had a much more significant effect on enhancing ADCC then [sic] the presence of bisected GlcNAc ... ." Office Action at pages 5-6. Thus, the Office contends that "it is unpredictable as to what level of bisecting GlcNAc will increase ADCC of any given antibody and indeed, each antibody would have to be tested empirically to determine whether the claimed bisected glycan ratio increases or decreases ADCC." *Id.* at page 6. Applicants respectfully disagree with these assertions.

First, Applicants emphasize that the claims recite a method for producing a recombinant antibody having increased Fc mediated cellular cytotoxicity or increased Fc receptor binding activity comprising *glycoengineering a host cell* so that it *has an altered level of activity of at least one glycoprotein-modifying glycosyltransferase.* Applicants agree that, as shown in the present specification, ADCC activity for C2B8 antibodies expressed in CHO cells that were glycoengineered to express GnTIII did, indeed, correlate with the level of Fc-associated bisected oligosaccharides. *See, e.g.*, Specification at page 9, lines 31-32. However, as discussed in more detail below, exogenous expression of GnTIII (which results in an increase in bisecting GlcNAc) is only one of the numerous possible ways taught by Applicants' disclosure to alter the level of activity of at least one glycoprotein-modifying glycosyltransferase. *See, e.g.*, discussion, *infra.* Thus, the Office is impermissibly reading limitations from the specification into all of the claims, even though, for example, independent claims 86 and 87 do not recite these limitations.

Second, Applicants respectfully assert that Shinkawa *et al.* do not show unpredictability in the art or that the role of bisecting GlcNAc in enhancing ADCC is "controversial." In fact, the findings of Shinkawa *et al.* are completely consistent with

the present specification. When Shinkawa *et al.* examined ADCC activity of anti-CD20 antibodies produced in LEC10 cells—a CHO mutant variant cell line that, unlike wild-type CHO cells, expresses GnTIII—as compared to anti-CD20 antibodies produced by a non-GnTIII-expressing CHO cell line, they did, in fact, report a concomitant increase in ADCC. Shinkawa *et al.* at 3470, first column. They noted that the antibodies produced by LEC10 cells had 74% bisecting GlcNAc and showed several-fold enhancement in ADCC activity compared to Rituxan® antibodies, which had 0% bisecting GlcNAc. *Id.* Shinkawa *et al.* explicitly recognized that, "[t]hese results suggest that a relatively high content of bisecting GlcNAc (74%) improves ADCC when IgG1 has a high content of fucosylated oligosaccharides...". *Id.* This finding is completely consistent with the present specification, which, as acknowledged at page 5 of the Office Action, showed that co-expression of a recombinant anti-CD20 antibody and exogenous GnTIII in CHO cells results in increased ADCC activity. *See* Specification at page 42, line 32 to page 43, line 18 and at Figure 15.

Third, the present specification is consistent with Shinkawa *et al.*, in that the specification explicitly recognizes that a decrease in fucosylated N-linked oligosaccharides, to which Shinkawa attributed an increase in ADCC, is associated with altered GnTIII expression:

> Higher accumulation of non-fucosylated (m/z 1664) bisected hybrid by-products, instead of fucosylated ones (m/z 1810), would agree with the fact that *oligosaccharides which are first modified by GnTIII can no longer be biosynthetic substrates for core α1,6-fucosyltransferase.*

Specification at page 38, lines 2-6 (emphasis added). Thus, well before Shinkawa *et al.*, the present specification explicitly identified a relationship between decreased core fucosylation of N-linked oligosaccharides in the Fc region and expression of GnTIII,

which results in an increase in ADCC. Likewise, the priority document (U.S. Provisional Application No. 60/082,581, incorporated by reference in its entirety into the present application), explicitly states that "[t]he addition of fucose to the core of oligosaccharides can take place at any point after reaction 5 of the [central reaction network of the N-linked glycosylation pathway], but it is also blocked by the modifications that *GalT or GnTIII* introduce." Exhibit 5, Priority Document at page 17, lines 28-30 (emphasis added). Thus, the priority document not only identifies the decreased fucose, but also that the decrease can be achieved by multiple enzymes.

The Office further contends that Shinkawa's results are "in contrast with the results presented by Applicants with the chCE7 antibody where high levels of bisecting GlcNAc actually reduced ADCC," and that each antibody would have to be tested to determine whether ADCC is increased or decreased as a result of glycoengineering. Office Action at page 6. Applicants respectfully point out that "[e]nablement is not precluded by the necessity for some experimentation, such as routine screening." *Wands*, 858 F.2d at 737. First, the specification expressly provides disclosure for how to determine optimal expression levels of a glycosyltransferase by using routine methods that are well-known in the art. Specification at page 14, lines 7-29. Second, Applicants respectfully submit that testing ADCC activity is routine in the art. The specification as filed provides detailed methods for testing ADCC activity. *See* Specification at page 33, line 33 to page 34, line 15. Furthermore, methods for assaying Fc-mediated cellular cytotoxicity were generally well known in the art. *See, e.g.,* Specification at page 42, lines 1-5 (citing Brunner *et al.*, 1968, *Immunology* 14:181-189). Thus, Applicants

respectfully submit that this basis for asserting that the unpredictability in the art would lead to undue experimentation is incorrect.

b) The Choice of Host Cell and Glycosylation Machinery do not Introduce Unpredictability

The Office contends that "with regard to the host cell used, it is unpredictable whether any particular change to the glycosylation machinery of said cell will have any effect on the glycosylation of any particular antibody so as to increase the ADCC of said antibody." Office Action at page 6. The Office cites Raju *et al.*, *Glycobiology* 10: 477-86 (2000), for the proposition "that different cell lines have different glycosylation machinery and that glycosylation of recombinant antibodies produced in different cell lines from different species will likely be significantly different." Office Action at page 5. The Office asserts that Applicants have not provided any teachings on what additional cell lines can be used to produce antibodies with increased ADCC or how the glycosylation machinery of the cells can be modified to enhance ADCC. *Id.* Applicants respectfully disagree with these assertions.

First, Applicants point out that "[t]he specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public." MPEP § 2164.05(a) (citing, *inter alia*, *In re Buchner*, 929 F.2d 660 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d 1367 (Fed. Cir. 1986). Here, the amended claims recite that the method is performed using *mammalian* host cells. As such, a detailed listing of every mammalian cell type is not required because mammalian cells are known in the art. Moreover, it was known in the art that mammalian cell types share common features of the N-linked glycosylation pathway. For example, Jenkins *et al.* provide a table summarizing major

glycosylation characteristics of different cell types. Jenkins *et al., Nature Biotech.* 14: 975, 976 (1996) (of record as Document AS19, and attached hereto as Exhibit 1). Therein it is shown that, of the various mammalian cell types tested (*e.g.*, hamster, mouse, rat, goat & sheep, and human), most show the same types of N-linked glycosylation (*e.g.*, oligo-mannose and complex glycosylation are present, but not hyper-mannose glycosylation). In fact, all mature mammalian N-linked oligosaccharides share the same common core structure (Man_3GlNAc_2), are formed by adding residues from the same pool of common oligosaccharide structures, and are joined to the common core oligosaccharide structure in predictable and well-defined configurations to form mature N-linked oligosaccharides. *See* Jenkins, Exhibit 1 at 975-76; *see also* Specification at Figure 1 (depicting the typical N-linked oligosaccharide structures). Furthermore, the glycoprotein-modifying glycosyltransferase enzymes (*e.g.*, ManI, ManII, GalT, GnTI, GnTII, GnTIII, GnTIV, GnTV, fucosyltransferase, and sialyltransferase) were known in the art and their mechanisms of action (*e.g.*, the oligosaccharide that is added by each glycosyltransferase and the structure to which it is added) have been extensively characterized. *See, e.g.,* Schachter, H., *Biochem. Cell Biol.* 64: 163-81 (1986) (of record as Document AS36 and attached hereto as Exhibit 2). Thus, there is predictability with respect to these features of the glycosylation mechanisms and because they are well-known in the art, the details do not need to be included in the specification.

The amended claims also recite that the Fc region containing N-linked oligosaccharides is an *immunoglobulin G* (IgG) Fc region. Glycosylation in the IgG Fc region occurs at a single conserved asparagine amino acid residue: Asn297 in the C_H2 domain. *See, e.g.*, Specification at page 21, lines 19-20. One of ordinary skill would have known that "[t]he Fc glycosylation site is a conserved feature for all mammalian

IgGs investigated..." *See, e.g.*, Jefferis and Lund, *Chem. Immunol.* 65: 111-128, 113 (1996) (attached hereto as Exhibit 3). Since the N-linked oligosaccharides in the Fc region are all attached to Asn297 (or the residue that corresponds thereto when the Fc region comprises an IgG fragment), the point of attachment for the core N-linked oligosaccharide structure does not vary at all, and therefore is completely predictable with respect to every antibody produced according to the claimed method.

Second, although the Office cites Raju *et al.* as evidence of unpredictability associated with choice of host cells and glycosylation machinery, Applicants respectfully disagree with the conclusion drawn from this reference. Raju *et al.* reported the differences in *endogenous* glycosylation patterns of proteins produced in cells from different animal species. This is not disputed. However, the present invention is directed to *glycoengineering* a mammalian host cell so that it has an *altered level of activity of at least one glycoprotein-modifying glycosyltransferase* and produces antibodies with preferred glycoforms. Thus, according to the present invention, a host cell that does not, in the absence of glycoengineering, produce recombinant antibodies having increased ADCC and/or Fc-mediated cellular cytotoxicity, can be glycoengineered to produce such antibodies. Indeed, the methods of the present invention overcome precisely the problem that Raju *et al.* identified; *i.e.*, finding a host cell that produces desired immunoglobulin glycoforms. Furthermore, Raju *et al.* reinforce what was evident from the articles by Jenkins *et al.* and Schachter; namely, that methods of characterizing glycosylation profiles from various cell types were well known and routine in the art.

Since it was known which enzymes facilitate glycosylation, which oligosaccharides they add, the configurations in which the oligosaccharides are added,

and the site to which they are attached, and since the glycosylation profile of any cell could be determined by routine methods and in view of the direction provided by Applicants' disclosure regarding structures for achieving increased Fc-mediated cytotoxicity and/or Fc receptor binding, Applicants respectfully submit that there was sufficient predictability in the art to practice the claimed invention without undue experimentation.

2. Applicants' Disclosure Provides Ample Guidance and Examples to Practice the Invention Without Undue Experimentation

With respect to the number of working examples provided in the specification, the Office recognizes that "Applicants present two examples of antibodies with enhanced ADCC produced in CHO cells transformed with a tet-regulated GnTIII gene." Office Action at page 6. With respect to the amount of guidance provided, the Office asserts that "[a]side from the two examples, applicants provide no direction for selecting cell lines, glycoengineering said cells so as to increase the ADCC for any given antibody by increasing the ratio of bisected, hybrid to bisected, complex glycans. The relevant art ... teaches that ADCC is not associated with this ratio, and that antibodies bearing this ratio may or may not have increased ADCC." *Id.* The Office therefore concludes that "[t]he specification requires the skilled artisan to practice trial and error experimentation with different antibodies, cell lines, and glycoprotein modifying glycosyltransferases to determine which will yield antibodies with enhanced ADCC activity." *Id.* Applicants respectfully disagree.

First, Applicants again emphasize that the "relevant art" referred to by the Office (*i.e.*, Shinkawa *et al.*) does not contradict what is described in the present specification, and, in fact, reinforces what was first taught in Applicants' disclosure. *See* discussion of

Shinkawa *et al.* in Section IV.A..1.a, *supra*. Second, it is well settled that "[t]he specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation." MPEP § 2164.02 at 2100-195 (citing *In re Borkowski*, 422 F.2d 94 (CCPA 1970). Furthermore, 'because only an enabling disclosure is required, applicant need not describe all actual embodiments." *Id.* In the present case, as acknowledged by the Office, Applicants do provide working examples, even though they are not required. However, the disclosure is not limited to the examples. "How a teaching is set forth, by specific example or broad terminology, is not important." M.P.E.P. § 2164.08 at 2100-198 (citing *In re Marzocchi*, 439 F.2d 220, 223-24, 169 USPQ 367, 370 (CCPA 1971)).

For instance, the present specification provides ample disclosure regarding suitable mammalian host cells for use in the present invention, including CHO cells, BHK cells, NS0 cells, SP2/0 cells, and hybridoma cells. Specification at page 15, lines 25-32. Applicants also identify numerous routes for engineering the host cells. For example, the specification describes transforming or transfecting a host cell with a nucleic acid molecule comprising a gene encoding a glyocsyltransferase; engineering a host cell to alter expression of a glycosyltransferase by introducing a promoter element that changes the expression of an endogenous enzyme; and the use of gene knockout technologies or ribozyme methods to alter the expression levels of a glycosyltransferase in the host cell. *Id.* at page 2, line 30 to page 13, line 1; page 15, lines 6-13; and page 15, lines 22-24.

The present specification also identifies numerous glycosyltransferases of which the activity may be altered, including GnTIII, GnTV, GalT, and ManII, as well as combinations of the glycosyltransferases. *Id.* at page 15, lines 18-30. The specification

also describes how to determine optimal expression levels for each glycosyltransferase by routine methods, including Western blot, Northern blot, lectin binding assays, and reporter genes. *Id.* at page 14, lines 7-29. Furthermore, the present specification describes how to measure ADCC activity and how to perform oligosaccharide analysis of an antibody, as well as providing citations to literature describing these methods. *Id.* at page 33, line 5 to page 34, 15. In view of what was already known to one of ordinary skill in the art, Applicants' disclosure, including the examples, provides sufficient guidance to practice the claimed invention without undue experimentation.

As further evidence that the specification provides sufficient guidance to practice the claimed invention, Applicants submit herewith the Declaration Under 37 C.F.R. §1.132 of Dr. Pablo Umaña ("the Declaration"). *See* Exhibit 4. The Declaration provides data showing conclusively that following the methods taught in the present specification results in recombinant antibodies having increased Fc mediated cellular cytotoxicity and/or increased binding to Fc receptors. *See* Declaration and Exhibits A-D appended thereto. Dr. Umaña is currently Chief Scientific Officer and Member of the Board at GlycArt Biotechnology AG in Zurich, Switzerland. He is an expert in the fields of molecular biology and immunology, with particular expertise in the area of antibody engineering, as evidenced by his *curriculum vitae* attached to the Declaration. *See* Declaration at § 2.

As noted by Dr. Umaña, the present application teaches that glycoengineered antibodies having increased binding affinity for Fc receptors and increased Fc-mediated cellular cytotoxicity can be obtained, according to one method, by engineering a host cell to coexpress an antibody and a glycoprotein modifying glycosyltransferase. (*See* Declaration at § 5.) GnTIII and ManII are specifically identified in the present

specification as useful glycoprotein modifying glycosyltransferases for this purpose. (*See* page 13, lines 18-30; page 21, lines 15-25.) In one embodiment, the present specification teaches that glycoengineered antibodies having increased Fc receptor binding affinity and increased Fc-mediated cellular cytotoxicity can be obtained by coexpressing the antibody with multiple glycoprotein-modifying glycosyltransferases, *e.g.*, GnTIII + ManII. (*See* Declaration at § 5.)

Exhibits B, C, and D to the Declaration disclose studies conducted after the present application, in which antibodies coexpressed with wild-type human mannosidase II ("hManII"), wild-type β(1,4)-N-acetylglucosaminyltransferase ("GnTIII"), and hManII + GnTIII, respectively, were assayed for ADCC and Fc receptor binding affinity. In each instance, glycoengineered antibodies exhibited significantly increased ADCC and Fcγ receptor binding compared to non-glycoengineered antibodies. (*See* Declaration §§ 6-19.) As noted by Dr. Umaña, this evidence demonstrates that practicing the techniques taught in the present application results in glycoengineered antibodies that exhibit enhanced Fc receptor binding and Fc-mediated cellular cytotoxicity according to the claimed methods. Thus, contrary to the Office's contention, the present specification does, indeed, provide sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention without undue experimentation.

3. Evaluation of the Other **Wands** *Factors Indicates that the Claimed Invention is Enabled*

a) State of the Art, Level of Skill, and Nature of the Invention

The Office asserts that the state of the art is undeveloped, and that the nature of the invention is complex and unpredictable. Office Action at pages 6-7. The Office acknowledges that the level of skill in the art is high, but asserts that, "given the

unpredictability of the art, the poorly developed nature of the art, the lack of guidance and the broad scope of the invention, it must be considered that the skilled artisan would have had to have conducted essentially trial and error experimentation in order to practice the claimed invention." *Id.* at page 7. Applicants agree that the level of skill in the art is high, but disagree with the Office's assessment that the state of the art and nature of the invention are unpredictable. As discussed in detail above with respect to the level of predictability in the art, the state of the art was well developed with respect to which enzymes facilitate glycosylation in mammalian cells, which oligosaccharides they add, the configurations in which the oligosaccharides are added, and the site to which they are attached in the IgG Fc region. Also well known were methods for determining glycosylation profiles and measuring ADCC activity. *See, supra.*

This knowledge in the art, coupled with the level of skill in the art, and in view of Applicants' disclosure provides more than sufficient guidance to one of ordinary skill in the art to practice the claimed invention without undue experimentation. Applicants also direct the Office to the Declaration of Dr. Umaña, which provides evidence that the claimed invention can be practiced successfully by one of ordinary skill in the art using the methods described in the present specification. Declaration, Exhibit 4. *See also*, discussion of Declaration, *supra*. Thus, considering other *Wands* factors such as the state of the art, the nature of the invention, and the level of skill in the art, the claimed invention is sufficiently enabled.

b) Scope of the Invention

The Office further asserts that "the scope of the invention is broad with the broadest claims encompassing producing any antibody having increased ADCC by providing any host cell from any species, glycoengineering said host cell to alter the

activity of any of the glycoprotein modifying glycosyltransferases and culturing the cell so as to produce the modified antibody." Office Action at page 7. Applicants respectfully disagree.

The amended claims are directed to a method for producing a recombinant antibodies comprising *an IgG Fc region* in *mammalian cells*. As set forth in detail above and below, with respect to written description, the amino acid residue in the IgG region to which N-linked oligosaccharides attach is the same for every antibody: Asn297. Also, mammalian cells were well known and characterized in the art. Furthermore, there are not an unlimited number of glycoprotein modifying glycosyltransferases, and a large majority of them had been well studied and characterized at the time the present application was filed. *See, e.g.,* Jenkins *et al.*, Exhibit 1, and Schachter, Exhibit 2. Therefore, Applicants respectfully submit that the claims are not unduly broad and that, in view of the knowledge in the art and the guidance provided in Applicants' disclosure, the full scope of the invention is enabled such that one of ordinary skill in the art could practice the claimed invention without undue experimentation.

4. Summary

For the above reasons, Applicants respectfully submit that a proper analysis of the *Wands* factors shows that Applicants' disclosure would allow one of ordinary skill in the art to practice the claimed invention without undue experimentation. As such, the claims are supported by an enabling disclosure. Applicants therefore respectfully request that the rejection under 35 U.S.C. § 112, first paragraph for lack of enablement be reconsidered and withdrawn.

B. Rejection Under 35 U.S.C. § 112, First Paragraph--Written Description

At page 8 of the Office Action, the Office indicates that claims 86-132 and 158-160 are rejected under the first paragraph of 35 U.S.C. § 112 for allegedly failing to comply with the written description requirement. The Office asserts that "[t]he claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." *Id.* Applicants respectfully traverse this rejection.

To meet the written description requirement, an applicant's disclosure must convey with reasonable clarity to one skilled in the art that, at the time of filing the application, the applicant was in possession of the claimed invention. *See Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64 (Fed. Cir. 1991); *In re Wertheim*, 541 F.2d 257, 262 (C.C.P.A. 1976); *In re Smythe*, 480 F.2d 1376, 1382 (C.C.P.A. 1973). Possession is shown "by such descriptive means as words, structures, figures, diagrams, formulas, etc. that fully set forth the claimed invention." *Lockwood v. Am. Airlines, Inc.*, 107 F.3d 1565, 1572 (Fed. Cir. 1997). However, "the invention claimed does not have to be described *in ipsis verbis* in order to satisfy the [written] description requirement of § 112." *In re Lukach*, 442 F.2d 967, 969 (C.C.P.A. 1971); *see also Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323 (Fed. Cir. 2000).

Likewise, an adequate written description does *not* require examples or actual reduction to practice, and "there is no *per se* rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure." *Falkner v. Inglis*, 448 F.3d 1357, 1366 (Fed. Cir. 2006). Furthermore, "[t]he descriptive text needed to meet [the written description requirement] varies with

the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence." *Capon v. Eshhar,* 418 F.3d 1349, 1357. There is no need to re-describe what is already known. *See id.*

For the reasons set forth below, the disclosure of the present application provides ample written description support such that one of ordinary skill in the art would have understood that Applicants had possession of the claimed invention at the time the application was filed.

1. The Claims are Directed to a Finite and Well-Defined Genus of Methods for Producing Antibodies

The Office alleges that the claims "read on a genus of methods where *any* cell can be used and *any* activity of *any* glycoprotein-modifying glycosyltransferase. . .can be altered so as to produce *any* antibody with enhanced ADCC." Office Action at page 9 (emphasis added). Thus, the Office appears to suggest that the claims encompass a vast and widely variant genus. The Office also asserts that Applicants have not provided sufficient number of representative species, stating that "the disclosure of two examples of antibodies having enhanced ADCC prepared using CHO cells is not a sufficient number to describe the claimed genus." *Id.* at page 10. Applicants respectfully disagree with these assertions.

The written description requirement for a genus can be satisfied by sufficiently describing a representative number of species. *See* MPEP § 2163 (Rev. 3, Aug. 2005) at 2100-182. "Satisfactory disclosure of a 'representative number' depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by members of the genus in view of the species disclosed." *Id.* at 2100-183. In determining whether written

description for a genus is sufficient, the knowledge of one skilled in the art must be considered. *Bilstad v. Wakalopulos*, 386 F.3d 1116, 1126 (Fed. Cir. 2004). Furthermore, because a patent specification is written for one of skill in the art, "[a] claim will not be invalidated on section 112 grounds simply because the embodiments of the specification do not contain examples explicitly covering the full scope of the claim language." *Faulkner*, 448 F.3d at 1366 (quoting *LizardTech, Inc. v. Earth Resource Mapping, PTY, Inc.*, 424 F.3d 1336, 1345 (Fed. Cir. 2005)). Applicants respectfully submit that, in the present case, the Office has not properly considered the knowledge in the art regarding glycosylation in mammalian cells. *See id.*

For the reasons set forth below, Applicants respectfully submit that the genera of host cells, IgG Fc regions, oligosaccharide structures, and glycosyltransferases encompassed by the claimed invention are not vast or widely variant and that, given the knowledge in the art, their members would be identifiable and recognizable to one of ordinary skill in the art based on the information provided in the specification as filed. *See, e.g., Amgen Inc. v. Hoechst Marion Roussel Inc.*, 314 F.3d 1313 (Fed. Cir. 2003). Also, considering the knowledge in the art, it is not necessary to provide examples or show an actual reduction to practice of every embodiment of the claimed invention. See, *Faulkner*, 448 F.3d at 1366; *see, also, Bilstad,* F.3d at 1126. Hence, Applicants have described a representative number of species within the claimed genus.

a) The Genus of Mammalian Host Cells is Well-Defined and Recognizable to One of Ordinary Skill in the Art

The Office Asserts that "[n]either applicants nor the prior art disclose additional host cells capable of increasing the ADCC of an antibody by glycoengineering the host cell..." Office Action at page 10. Applicants disagree. The amended claims recite

providing a *mammalian* host cell. The specification provides numerous examples of mammalian host cells, and the members of the genus of mammalian host cells could be readily envisioned by one of ordinary skill in the art.

By knowing that the host cell type is derived from a mammalian source, as recited in the claims, one of ordinary skill in the art would know that the cell type has features in common with other mammalian cell lines; in particular, the mammalian cell's ability to glycosylate proteins in a particular and defined manner. For example, as discussed above with respect to enablement, Jenkins *et al.* provide a table summarizing major glycosylation characteristics of different cell types and showing that, of the various mammalian cell types tested (*e.g.*, hamster, mouse, rat, goat & sheep, and human), most have the same types of N-linked glycosylation (*e.g.*, oligo-mannose and complex glycosylation are present, but not hyper-mannose glyocsylation). Jenkins *et al.*, Exhibit 1. Thus, the members of the genus of mammalian cell types encompassed by the claimed invention have common features that could be recognized and identified by one of ordinary skill in the art, and which make this a well-defined genus.

In addition, the specification as filed describes several exemplary mammalian host cell lines. In particular, the specification states that, "[p]referably mammalian cells are used as host cell systems ..." Specification at page 17, lines 29-30. Furthermore, the specification provides that, "[m]sot preferably, CHO cells, BHK cells, NS0 cells, or SP2/0 cells, or alternatively, hybridism cells are used as host cell systems." *Id.* at lines 32-33. Thus, the specification contemplates the use of any type of mammalian cells in accordance with the claimed invention, and expressly provides a representative number of species of mammalian cell types that may be used in accordance with the claimed invention.

A similar situation was addressed by the Federal Circuit in *Amgen*, which held that disclosure of only two species of vertebrate or mammalian cells provided adequate written description support for the entire genus of vertebrate or mammalian cells used to produce glycosylated erythropoietin according to the claimed invention. 314 F.3d at 1398. The court stated that "the words 'vertebrate' and 'mammalian' readily 'convey[] distinguishing information concerning [their] identity' such that one of ordinary skill in the art could 'visualize or recognize the identity of the members of the genus.'" *Id.* (citations omitted). Given that multiple species within the genus of mammalian cell types are explicitly disclosed in the present specification--and certainly more than two as in *Amgen*--and because mammalian cell types share common glycosylation machinery and characteristics that were known in the art, Applicants respectfully submit that a representative number of species was provided such that one of ordinary skill in the art could envision the members of the claimed genus.

b) The Genus of Glycosyltransferases is Finite and Well-Characterized

The Office asserts that "[n]either applicants nor the prior art disclose any non-GnTIII glycosyltransferase which has been engineered to increase the ADCC of any antibody in any cell," and suggests that, "[g]iven the ambiguity in what oligosaccharide features are actually important in determining ADCC, the skilled artisan would not be able to envision additional embodiments of the claimed invention." Office Action at page 10. Applicants respectfully disagree.

The genus of glycoprotein-modifying glycosyltransferases that can be used according to the claimed methods is not infinite or widely variant. For example, as discussed in the priority document, there are only eight Golgi-localized enzymes that are

responsible for the distribution of oligosaccharides into the major structural classes of oligosaccharides: ManI, ManII, GalT, GnTI, GnTII, GnTIII, GnTIV, and GnTV. *See, e.g.,* Priority Document at page 14, lines 25-27, page 16, line 4 to page and at Figure 2 (excerpts attached hereto as Exhibit 5). In addition, enzymes that catalyze terminal glycosylation reactions, such as fucosyltransferases, galactosyltransferases, and sialytransferases, were known in the art and their mechanisms of action have been characterized. *See, e.g.*, Youakim and Shur, *Annals New York Acad. Sci.* 745: 332-35 (1994) (attached hereto as Exhibit 6). Furthermore, the reactions catalyzed by known glycosyltransferases (*e.g.*, the oligosaccharide that is added by each glycosyltransferase and the structure to which it is added) have been characterized. *See, e.g.*, Priority Document at page 16, line 4 to page 17. *See also*, Schachter, Exhibit 2. Because the finite and well-defined genus of glycosyltransferases and their mechanisms of action were known in the art, there is no need to describe each one in detail. *See Capon*, 418 F.3d at 1357-58. Thus, the members of the genus could be envisioned by one of ordinary skill in the art.

(1) Exemplary Glycosyltransferases and Methods to Alter Their Activity are Provided by the Specification

The specification provides ample disclosure on how mammalian host cells may be glycoengineered according to the present invention, including providing numerous embodiments of the glycoprotein-modifying glycosyltransferases that can be used, as well as combinations of these glycosyltransferases. For example, the specification as filed states that "[t]he invention is contemplated to encompass engineered host cells expressing **any type of glycoprotein-modifying glycosyl transferase** as defined

herein." Specification at page 13, lines 18-19 (emphasis added). The specification also identifies that,

> in preferred embodiments, at least one glycoprotein-modifying glycosyl transferase expressed by the host cells of the invention is **GnT III, or, alternatively, β(1,4)-N-acetylglucosaminyltransferase V (GnT V).** However, also other types of glycoprotein-modifying glycosyl transferase may be expressed in the host system, typically in addition to **GnT III or GnT V, including β(1,4)-galactosyl transferase (GalT), and mannosidase II (Man II).** In one embodiment of the invention, **GnT III is coexpressed with GalT.** In another embodiment of the invention, **GnT III is coexpressed with Man II**. In a further embodiment of the invention, **GnT III is coexpressed with GalT and Man II.** However, any other permutation of glycoprotein-modifying glycosyl transferases is within the scope of the invention. Further, expression of a glycosidase in the host cell system may be desired.

Id. at page 13, lines 20-30 (emphasis added).

In addition, as discussed above with respect to enablement, Applicants also identify numerous routes for engineering the host cells to alter the expression of a glycosyltransferase. For example, the specification describes transforming or transfecting a host cell with a nucleic acid molecule comprising a gene encoding a glyocsyltransferase; engineering a host cell to alter expression of a glycosyltransferase by introducing a promoter element that changes the expression of an endogenous enzyme; and the use of gene knockout technologies or ribozyme methods to alter the expression levels of a glycosyltransferase in the host cell. *Id.* at page 2, line 30 to page 13, line 1; page 15, lines 6-13; and page 15, lines 22-24. The specification also describes how to determine optimal expression levels for each glycosyltransferase by routine methods, as well as how to measure ADCC activity and how to perform oligosaccharide analysis of an antibody. *Id.* at page 14, lines 7-29; and at page 33, line 5

to page 34, 15. Clearly, the specification as filed contemplates multiple paths to achieve the claimed methods.

(2) The Level Knowledge in the Art was High

The Office refers to the alleged unpredictability of whether altering the activity of any glycosyltransferase besides GnTIII (in CHO cells) can result in increased ADCC activity. *Id.* at page 9. Applicants respectfully disagree. It was already shown above with respect to enablement that the art was not unpredictable as suggested by the Office. Furthermore, there was knowledge in the art to indicate that it is possible to manipulate the glycosylation machinery in cells of various types to achieve a preferred glycosylation profile. For example, Grabenhorst *et al., Glycoconjugate J.* 16: 81-97 (1999) (attached hereto as Exhibit 7), which published shortly after the filing date of the present application, comments on studies in glycosylation for the preceding decade, and describes the advances in the art to that point. *See id.* at page 82, column 1.

Grabenhorst *et al.* recognized that, "...during the past 12 years much work has been published on the *structural characterization* of recombinant glycoproteins expressed from various *mammalian and nonmammalian expression systems*." *Id.* (emphasis added). They further stated that "a great deal of efforts [sic] is presently going into attempts to improve recombinant host cell lines, and here especially, mammalian cells, for the manufacturing of glycoprotein pharmaceuticals ... with novel *in vivo* properties." *Id.* Based on their observations, they provide a model for

> the recombinant expression of the full length form of human glycosyltransferases along with a suitable reporter glycoprotein (here human β-TP) at a constant epxpression level in a heterologous mammalian host cell line that is devoid of the pertinent enzyme activity. **This is considered to represent a valuable model and should enable the comparison of the *in vivo* specificities of different members of a glycosyltransferase family [citations omitted] and allow the selection**

> **of the optimal enzyme suitable for the glycosylation engineering of host cell lines** for the production of a new generation of glycotherapeutics with defined altered glycosylation characteristics.

Id. at 87 (emphasis added).

With respect to knowledge of mammalian host cells used for recombinant expression of human therapeutic glycoproteins, Grabenhorst *et al.* state that "over the past 12 years the literature reporting on the glycosylation analysis of recombinant glycoproteins from different hosts has accumulated tremendously." *Id.* at page 82, column 1. They also provide a table summarizing the structural features of N-linked oligosaccharides from recombinant glycoproteins expressed in different types of mammalian host cells (*i.e.*, hamster cell lines CHO and BHK-21, and murine cell lines C127 and Ltk-). *Id.* at Table 1.

Grabenhorst *et al.* also recognized that

> [s]ignificant advances in the sensitivity of carbohydrate structural analysis has [sic] been achieved during the past three years. Especially in mass spectrometry (on-line ESI-MS, nanospray tandem mass spectrometry (ESI-MS/MS) and improved MALDI/TOF techniques), very sensitive instrumentation for glycosylation analysis has been made available to a broader group of research units, and thus has led to a broader use of complementary tools by academic researchers and in industrial laboratories.

Id. at page 83, col. 1.

Thus, Grabenhorst *et al.* emphasize the fact that there was knowledge in the art regarding the manipulation of glycosylation machinery to achieve preferred glycoprotein glycoforms from which models could be developed, and that the tools for such

manipulation (cell types, glycosylation structures, detection methods, etc.) were also known in the art.

(3) The Priority Document Shows that the Claimed Invention is More than Adequately Described

As further evidence that the claimed invention has sufficient written description support, Applicants point to the priority document, which provides a detailed description of a physical model for determining heterogeneity of glycoforms that result from processing in the N-linked glycosylation pathway. *See* Priority Document at Figure 2; at page 3, lines 26-31; at page 16, line 4 to page 18, line 20. In describing the physical model, the priority document explicitly recognizes that widely-used mammalian industrial cell lines such as CHO cells and BHK cells share common features of the central reaction network in the N-linked glycosylation pathway. *Id.* at page 17, lines 9-22.

In describing the physical model of glycosylation, the priority document specifically recognizes that "[t]he addition of fucose to the core of oligosaccharides can take place at any point after reaction 5 of the [central reaction network of the N-linked glycosylation pathway], but it is also blocked by the modifications that *GalT or GnTIII* introduce." Priority Document at page 17, lines 28-30 (emphasis added). Expressing GalT or GnTIII would therefore be expected to alter the fucosyltransferase activity. Thus, the priority document specifically provides additional ways to alter activity of a glycoprotein-modifying glycosyltransferase other than GnTIII.

The priority document also provides a detailed description of a mathematical model "to calculate the expected qualitative trends in the N-linked oligosaccharide distribution resulting from changes in the levels of one or more enzymes involved in the

network of enzyme-catalyzed reactions which accomplish N-linked oligosaccharide biosynthesis." *Id.* at page 12, lines 9-12. CHO cells were chosen as a specific example for the model "since CHO cells are currently the most common host for the industrial production of therapeutic glycoproteins." *Id.* at page 13, lines 6-7. However, as specifically recognized in the priority document, the "[v]alues for the parameters in the model and their normal ranges can either be found in the literature or estimated from literature information." *See id.* at page 12, line 14 to page 13, line 9.

These physical and mathematical models further support what is disclosed in the specification as filed; namely, that recombinant, glycoengineered antibodies with desired properties (*e.g.*, increased ADCC and/or increased Fc receptor binding) can be achieved by manipulating the expression of various enzymes in the N-linked glycosylation pathway in mammalian host cells. The choice of the enzymes to be manipulated for production of a preferred glycoform can be made based on the known activity of the enzymes (*e.g.* the oligosaccharide they will attach to a given residue on the core oligosaccharide structure) and the machinery that exists in the chosen mammalian host cell such that a desired glycosylation profile is achieved. These parameters are either known, or can be determined readily from the knowledge in the art. As such, the disclosure of the present application provides more than ample written description support.

c) The Point of N-linked Oligosaccharide Attachment Does Not Vary

The Office asserts that one of ordinary skill in the art would not be able to envision additional embodiments of the claimed invention because of an alleged "ambiguity" regarding the oligosaccharide features that are important in determining

ADCC activity, and an alleged diversity of antibodies involved. Office Action at page 10. Applicants respectfully disagree.

The amended claims recite that the Fc region containing N-linked oligosaccharides is an immunoglobulin G (IgG) Fc region. As discussed above, glycosylation in the IgG Fc region occurs at a single conserved asparagine residue, Asn297 in the C_H2 domain. *See, e.g.*, Specification at page 21, lines 19-20; Jefferis and Lund, Exhibit 3 at 113. One of ordinary skill in the art would have known that this glycosylation site is a conserved feature of mammalian IgGs. *Id.* Since the N-linked oligosaccharides in the Fc region are all attached to Asn297 (or the residue that corresponds thereto when the Fc region comprises an IgG fragment), the point of attachment for the core N-linked oligosaccharide structure does not vary at all, and one of ordinary skill in the art could easily envision other embodiments of an IgG Fc region containing N-linked oligosaccharides.

d) The Genus of Oligosaccharide Structures is Well-Defined and Finite

The genus of N-linked oligosaccharides encompassed by the claimed invention is also well-defined, having members that are known in the art and that have been extensively characterized. In particular, all mature N-linked oligosaccharides have the same common core structure, Man_3GlNAc_2, and all of the different glycoforms of a glycoprotein are based on the same pool of common oligosaccharide structures: mannose (Man), N-acetylglucosamine (GlcNAc), galactose (Gal), sialic acid (NeuAc), N-acetylgalactosamine (GalNAc), and fucose (Fuc). *See* Jenkins Exhibit 1 at 975-76. These common oligosaccharides are joined to the common core oligosaccharide structure in predictable and well-defined configurations to form mature N-linked oligosaccharides.

See id.; *see also* Specification at Figure 1 (depicting the typical N-linked oligosaccharide structures).

As a further example that the possible glycoforms encompassed by the present invention are finite and not widely variant, Jefferis and Lund show that, for a complex biantennary N-linked oligosaccharide structure in an IgG Fc region, there is only "a total of 36 structurally unique oligosaccharides [that] may be attached at each Asn297 residue." Exhibit 3 at 113. Thus, it is clear from the fact that N-linked oligosaccharides are based on a common core structure and the possible combinations of non-core oligosaccharides are derived from a common pool of sugar residues, the genus of possible N-linked oligosaccharide structures that can be added at the common Asn297 residue in the IgG Fc region is well-defined and finite and could be envisioned by one of ordinary skill in the art.

2. The Claims are Directed to Methods of Producing Antibodies with Common Structural Features

The Office asserts that "Applicants claim methods to produce antibodies with increased ADCC by glycoengineering by function only, without a correlation between structure and function" Office Action at page 9. Applicants respectfully disagree. First, a correlation between structure and function is not a per se requirement for an adequate written description. *See Faulkner,* 448 F.3d at 1366. Second, Applicants have provided a correlation between structure and function, even though such a showing is not required. In fact, the common structural features are explicitly recited in the claims. Specifically, the claims are directed to methods for producing 1) IgG antibodies 2) having an Fc region containing N-linked oligosaccharides 3) in mammalian host cells that have been

glycoengineered to have an altered level of activity of at least one glycoprotein-modifying glycosyltransferase.

The Fc region of IgG antibodies is well-characterized. As discussed in detail elsewhere herein, the oligosaccharide structures attached to the antibody Fc regions are likewise well-characterized. Finally, the claims are limited to methods of producing antibodies that have been glycoengineered to have an altered level of activity of at least one glycoprotein-modifying glycosyltransferase, which Applicants have demonstrated results in increased ADCC and Fc receptor binding. Thus, contrary to the Office's assertion, the claimed methods produce antibodies that all share readily identifiable structural features relating to the desired biological functions of increased ADCC and increased Fc receptor binding.

3. Summary

Given the knowledge in the art as discussed above, one of ordinary skill could visualize or recognize members of the genera of mammalian host cells, IgG Fc regions, glycosyltransferases, and oligosaccharide structures encompassed by the claimed invention. Furthermore, Applicants are not required to include in the specification all that was known in the art with respect to glycosylation in mammalian cells in order to provide sufficient written description. Rather, the specification as filed describes a representative number of species sufficient to indicate that the Applicants had possession of the claimed invention.

For the above reasons, Applicants respectfully submit that, in the present case, the written description requirement of 35 U.S.C. § 112, first paragraph is met. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

C. Rejection Under 35 U.S.C. § 112, First Paragraph--New Matter

The Office rejected claims 90, 94, 96, 97, 101, 108, 109, 114, 122, 127, and 128 under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the written description requirement by adding new matter. Office Action at page 10. Applicants respectfully traverse this rejection.

The Office alleges, in particular, that there is no support in the application as originally filed for claims directed to "a method for producing a recombinant antibody having increased ADCC comprising altering (i.e. decreasing) the activity of core α-1,6-fucosyltransferase or modifing the activity of the three enzymes GnTIII, α-mannosidase II and β(1,4)-N-acetylglucosaminyltransferase." *Id.* at pages 10-11. Applicants respectfully disagree.

As discussed, *supra*, the present specification provides that other glycosylation mechanisms can be altered, and in particular, that the activity of core α-1,6 fucosyltransferase can be decreased:

> Higher accumulation of non-fucosylated (m/z 1664) bisected hybrid by-products, instead of fucosylated ones (m/z 1810), would agree with the fact that oligosaccharides which are first modified by GnTIII *can no longer be biosynthetic substrates for core α1,6-fucosyltransferase.*

Specification at page 38, lines 2-6 (emphasis added). Clearly, if GnTIII blocks the activity of the core fucosyltransferase, its activity is decreased. Therefore, there is explicit support for claims directed to altering the activity of this enzyme.

In addition, the specification explicitly contemplates the use of glycosyltransferases other than GnTIII, including GnTV, GalII, and ManII, as well as the use of combinations of glycosyltransferases. *See* Specification at page 13, lines 18-30

(reproduced *supra*). Therefore, there is explicit support for claims directed to altering the level of activity of GnTIII, GnTV, GalT, and ManII.

The Office further contends that there is no support for claims to "a method for producing a recombinant antibody having increased ADCC wherein said antibody has an increased proportion of nonfucosylated oligosaccharides or wherein the predominant N-linked oligosaccharide is nonfucosylated or wherein the predominant N-linked oligosaccharide in the Fc region is not a high-mannose structure." Office Action at page 11. Applicants respectfully disagree.

As already discussed, the present specification indicates that, when GnTIII is expressed, it is no longer a substrate for the core fucosyltransferase. Specification at page 38, lines 2-6. Likewise, the priority document explicitly states that "[t]he addition of fucose to the core of oligosaccharides can take place at any point after reaction 5 of the [central reaction network of the N-linked glycosylation pathway], but it is also blocked by the modifications that *GalT or GnTIII* introduce." Priority Document at page 17, lines 28-30 (emphasis added). Thus, the priority document also identifies a relationship between GalT expression and a reduction in core fucosylation. If the core fucosyltransferase cannot add a fucose residue to the core oligosaccharide structure, there will be an increased proportion of nonfucosylated oligosaccharides.

With respect to producing an antibody wherein the predominant N-linked oligosaccharide is non-fucosylated, Applicants point to the MALDI-TOF peaks in Figure 9E. In particular, the peak identified as "m/z 1664" represents non-fucosylated structures. *See* Specification at Figure 9 and page 37, line 22 to page 38, line 6. Furthermore, embodiments of the invention that were clearly contemplated by the specification as filed do, indeed, result in antibodies with increased ADCC, wherein the

predominant N-linked oligosaccharide is non-fucosylated. For example, the specification as filed provides that "[i]n one embodiment of the invention, GnTIII is co-expressed with ManII." Specification at page 13, lines 26-27 (emphasis added). As shown by Dr. Umaña's Declaration and accompanying Exhibit D, when later tested, the co-expression of GnTIII and ManII resulted in antibodies wherein the predominant N-linked oligosaccharide is non-fucosylated. *See* Declaration of Dr. Pablo Umaña at page 5, §§ 18-21, and Exhibit D. For example, in Figure 2 of Exhibit D, the predominant MALDI-TOF peak is at m/z 1543, which represents a non-fucosylated oligosaccharide. Furthermore, the three MALDI-TOF peaks corresponding to non-fucosylated oligosaccharides (m/z 1339, 1543, and 1705) represent 70% of the total oligosaccharides. *See* Declaration of Dr. Pablo Umaña at page 5, § 18, and Exhibit D at Figure 2. Finally, the antibodies that were co-expressed with ManII and GnTIII displayed increased ADCC and increased Fc receptor binding compared to antibodies produced in non-engineered host cells. Declaration at page 5, §§19-21 and Exhibit D at Figures 3 and 4.

With respect to producing an antibody wherein the predominant N-linked oligosaccharide is not high mannose, the specification states that "[T]he peak at m/z 1257 is present at a level of 10-15% of the total in the CHO-derived samples ... (FIGURE 9). It corresponds to five hexoses plus two HexNAcs. The only known N-linked oligosaccharide structure with this composition is a five mannose-containing compound of the high-mannose type." Specification at page 38, lines 7-14. Clearly if the high mannose structures are present at a level of only 10-15% of the oligosaccharide structures in the glycoengineered CHO cells, then high mannose is not the predominant glycoform.

Therefore, contrary to the Office's assertions, claims 90, 94, 96, 97, 101, 108, 109, 114, 122, 127, and 128 are supported by the specification as originally filed and therefore do not constitute new matter. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

VII. Rejections for Obviousness-Type Double Patenting

The Office has rejected claims 86-88, 90-91, 97-98, 102-103, 106-107, 110-113, 120-121, 123-125, 129, 131-132, and 159-160 on the ground of non-statutory obviousness-type double patenting over claims 1-10 of U.S. Patent No. 6,602,684 and over claims 1-27 of co-pending Application No. 11/199,232. Office Action at page 12. Applicants respectfully request that these rejection be held in abeyance until otherwise allowable claims are identified, at which time Applicants will consider filing a Terminal Disclaimer.

VIII. Claim Objections

The Office has objected to claim 97 under 37 C.F.R. § 1.75(c) as allegedly being of improper dependent form for failing to further limit the subject matter of a previous claim. Office Action at page 13. Applicants respectfully traverse this objection.

Specifically, the Office contends that "[c]laim 97 is broader than the claims from which it depends because it recites that the activity is expression of at least one glycoprotein-modifying glycosyltransferase; however claim 97 is dependent on claim 90 which recites that the glycoprotein-modifying glycosyltransferase is selected from a group consisting of five specific glycosyltransferases." *Id.* (emphasis omitted). Applicants respectfully disagree.

Claim 97 is in proper dependent form. Claims 86 and 87 recite: "glycoengineering said host cell so that said host cell has an altered level of activity of at

least one glycoprotein-modifying glycosyltransferase." Claim 90 specifically recites a Markush group of five specific glycosyltransferases, but does not limit what the altered level of activity is. Claim 97 specifies that the altered level of activity is expression of the at least one glycosyltransferase as recited in claim 90 (e.g., as opposed to inhibition or some other alteration in the level of activity). Therefore, claim 97 does further limit the subject matter of claim 90 and is in proper dependent form.

Accordingly, Applicants respectfully request that the objection be reconsidered and withdrawn.

IX. Oath/Declaration

The Office indicates that the oath or declaration in the present case is defective because inventor James E. Bailey is deceased. Office Action at page 13. Applicants submit herewith a Supplemental Declaration signed by M. Sean Bailey, as legal representative of James E. Bailey, in accordance with 37 C.F.R. § 1.42.

Conclusion

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested. Applicants believe the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

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